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Mechanisms of transmission in the central nervous system

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My first duty, most willingly discharged, is to express my appreciation of being asked to give the Clover Lecture. I have to confess that my knowledge of Joseph Clover was mainly of his inhaler and that he trained and practised at the best medical school in London. It has been a pleasure to discover, with the help of Mr Lefanu and my own old University College Hospital Magazines, that he was an experimentalist, willing to undertake the discipline of designing apparatus as well as using it, of an attractive, gentle, yet evidently tenacious disposition, and one of whom friends – including such as Sydney Ringer – spoke in unusually affectionate terms. It is always interesting to wonder what great men of the past would have done if they lived today. Perhaps, as then, a clinical interest and skill in making and using apparatus might have directed him to anæsthetic research; or perhaps this bent was acquired when, as a medical student, he saw the first operation under ether in Britain, when Robert Liston amputated the leg of Frederick Churchill, a butler, in the leisurely time, by the anæsthetist's estimate, of twenty-eight seconds. I hope that today's subject might have been of interest to him; perhaps it might have been his chosen field.

Work on the central nervous system has particular interest for anæsthetists, since any major discovery in this field promises to have a speedy practical significance. From the spate of work in progress, one might imagine that in fact we are advancing rapidly in our understanding of such matters. In some respects this is true; we know much more about the activity of particular cells and about their functional connections, and we have a formidable array of drugs with which central nervous activity can be modified. But intense activity sometimes conceals ignorance. If one probes deeper with one's questions, into, say, the mechanism of action of an anæsthetic, one arrives with something of a shock at the discovery that we have no adequate idea how one nerve cell provokes or restrains the activity of another, although it is on these basic mechanisms that all the higher integrations are

The Joseph Clover Lecture, 1958

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built. Now before the days of chemical transmission this might not have been regarded as very serious; one could perhaps assume provisionally that the activation of one cell by another was accomplished by something like a telephone switchboard. But the development of our knowledge of chemical transmission has changed this. It has, of course, meant that synaptic events have an interest and subtlety beyond that of the spread of electrical currents. But more important, the conclusion that a chemical link intervenes between one nerve cell and the next, is crucial for therapeutics; for if this is so, drugs related to the natural chemical transmitters can be developed. This relevance of chemical transmission to therapeutics is not always explicitly recognised. But from the practical therapeutic point of view, one could argue that if we lacked the identification of acetylcholine and sympathetic amines as chemical transmitters in the periphery, we should also lack (for clinical use) muscle relaxants, many hypotensive agents, drugs like the anticholinesterases and carbachol, sympathomimetics and anti-adrenalines; and one cannot see how the line of research which led to antihistamines and tranquillizers could ever have started.

I propose, therefore, to discuss some of the information available about the way one neurone interacts chemically with another. But during the discussion it is important that we bear in mind constantly the sort of evidence we need to establish such an interaction. Thus, a chemical transmitter must be shown to be released specifically, when a specified nervous pathway is activated. The nervous tissue should contain the transmitter, and be able to synthesise it. There must be machinery for making it transient in action, lest successive signals get confused. Drugs should exist which can imitate, preserve, or block the transmitter, and these should have parallel effects on normal transmission. If evidence much short of this is accepted, we stand only to be led astray.

POSSIBLE CANDIDATES AS CENTRAL TRANSMITTERS

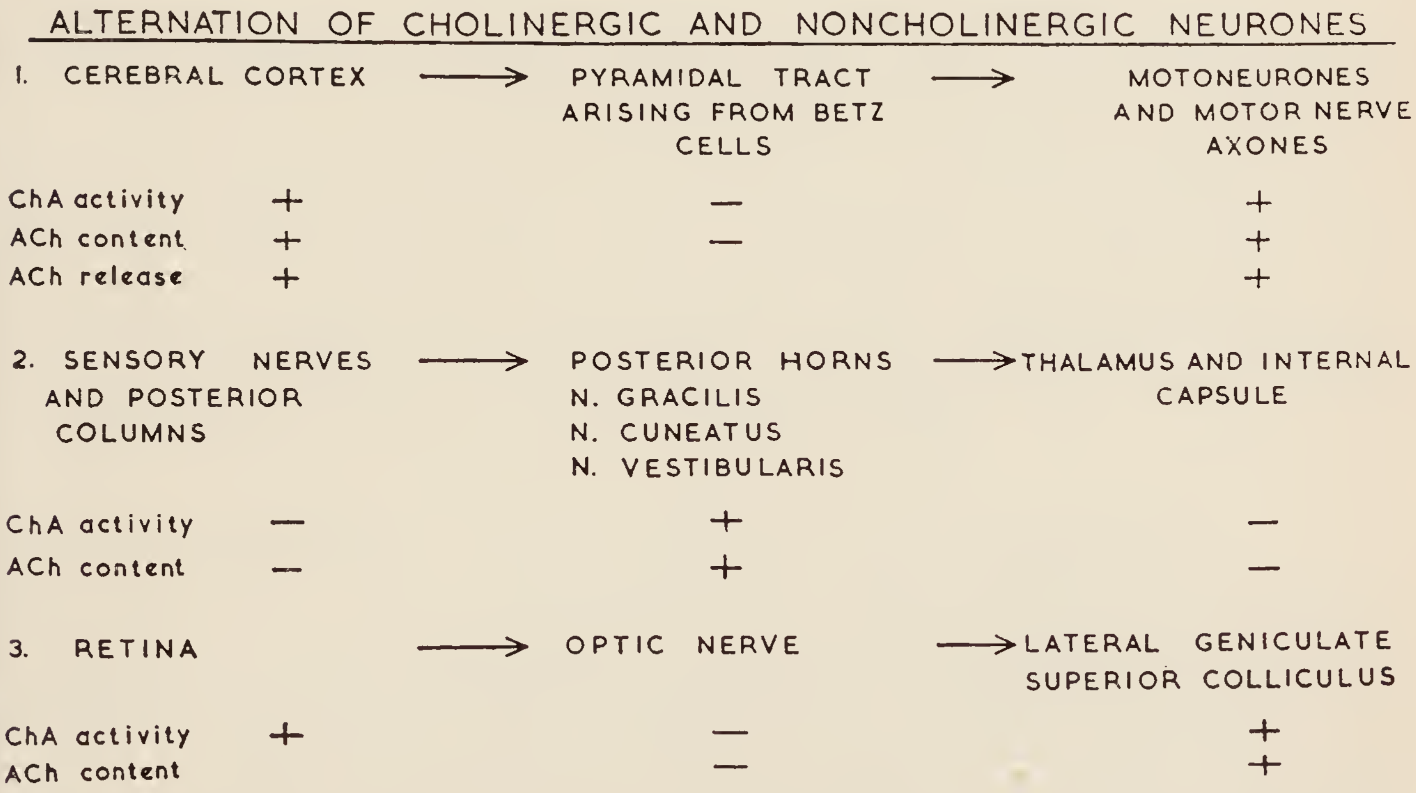
Of the sufficiently well characterised substances which we must consider as possible transmitters, I would like briefly to mention five. These are acetylcholine, noradrenaline, 'serotonin', Substance P, and γ-amino butyric acid.

(*a*) *Acetylcholine*

The discovery that acetylcholine played such an important role at peripheral synapses, including the autonomic ganglion, has prompted an extensive investigation as to its possible central role. This has been fully reviewed, especially by Feldberg[10] to whom we owe so much work in this field, and I wish only to mention briefly salient points. It

is clear that acetylcholine occurs in the spinal cord and in the brain. The enzyme which makes it, choline acetylase, also is widely distributed, as are the enzymes, cholinesterases, destroying it (although in a different manner). Acetylcholine accumulates if an anticholinesterase is given so that it is preserved. One of the nicest examples of this is the experiment by MacIntosh and Oborin[21] who placed little cups of Tyrode on the exposed cortex and found that if the fluid contained eserine, acetylcholine would accumulate in these cups, to an extent proportional to the intensity of electrical activity of the cortex underlying the cup. In addition, anticholinesterases themselves have dramatic effects, particularly if they have a chemical structure, as do DFP or eserine, which allows them to penetrate to the brain[3]. Drugs like atropine and hyoscine, which are recognized for their ability to antagonize acetylcholine's action peripherally, can exert central effects. There is, therefore, a very good case for acetylcholine being involved somewhere in the brain.

But if one comes down to the more specific question 'where?', the position is much more difficult. One of the most interesting attacks on this was by Feldberg and Vogt[11], who studied the regional distribution of choline acetylase, using the ability of a tissue to make acetylcholine as a sign that the cells in it could release acetylcholine. They found that there seemed an alternation of neurones in the central nervous system such that if one neurone could synthesise acetylcholine, then the following neurone tended to be unable to and was presumably not cholinergic. FIGURE 1 lists three of the alternations to which Feldberg



ALTERNATION OF CHOLINERGIC AND NONCHOLINERGIC NEURONES

1.	CEREBRAL CORTEX →	PYRAMIDAL TRACT ARISING FROM BETZ CELLS →	MOTONEURONES AND MOTOR NERVE AXONES
ChA activity	+	—	+
ACh content	+	—	+
ACh release	+		+
2.	SENSORY NERVES AND POSTERIOR COLUMNS →	POSTERIOR HORNS N. GRACILIS N. CUNEATUS N. VESTIBULARIS →	THALAMUS AND INTERNAL CAPSULE
ChA activity	—	+	—
ACh content	—	+	—
3.	RETINA →	OPTIC NERVE →	LATERAL GENICULATE SUPERIOR COLLICULUS
ChA activity	+	—	+
ACh content		—	+

FIG. 1 Diagram of possible alternations of cholinergic and non-cholinergic neurones. For each pathway, the nature of the evidence is indicated; *i.e.* the presence (+) or lack (—) of cholineacetylase activity, acetylcholine content, or acetylcholine release during activity.

and Vogt drew particular attention. Of these three, the first, that is, the sequence down the pyramidal pathway, is now suspect because of the evidence that the pyramidal tract does not directly innervate motoneurones but that there is another neurone intercalated before the anterior horn cell. But the sequence on the sensory side has been fully substantiated by recent work by Dr Hebb and her colleagues[15 17 18].

The significance of such an alternation of chemical transmitter is still puzzling. It would have been simple if one knew that a cell could not both release acetylcholine and be sensitive to it. But this is not the case. Parasympathetic ganglia, such as the ciliary ganglion, or those innervated by the vagus, are certainly activated by acetylcholine presynaptically, and certainly release acetylcholine at their endings. There is therefore no reason to suppose that there is any basic difficulty in neurones both responding to and releasing acetylcholine. Dr Hebb has suggested that the alternation between cholinergic and noncholinergic may be a particularly stable sequence, suitable as a reliable transmitter of sensory information to the centre, but insufficiently plastic for the more complex reactions higher in the brain and hence restricted to the sensory paths. Feldberg and Hebb have both pointed out that the development of the neocortex seems to be accomplished by the development of noncholinergic neurones: Feldberg has even suggested that the cholinergic neurone may be the primitive cell. These are fascinating ideas. But we must still note that a definite release of acetylcholine by specific cell groups, or a reproduction by acetylcholine of specific synaptic events, is still lacking at all synapses – save one, the rather specialised Renshaw cell in the spinal cord recently studied by Eccles, Eccles and Fatt[7] and Curtis, Eccles and Eccles[5] and deserving more discussion than we have time for. This cell is innervated by a recurrent collateral from the motor-axone and itself inhibits the motoneurones – a curious negative feed-back of obscure function. The cell responds, as does the motor endplate, to acetylcholine-like substances. But even though this is the only central synapse at which ACh can be confidently implicated, everyone must admit, I think, that acetylcholine has a wider central synaptic function to play, yet to be discovered.

(*b*) *Noradrenaline, hydroxytryptamine and Substance P*

Although these three compounds are rather different pharmacologically, one may provisionally group them together for our purposes today, because their central distributions are alike. *Noradrenaline* is familiar enough. Serotonin or *5-hydroxytryptamine* (5HT) is a more recent recruit to pharmacology, first appearing as a vaso-active substance in serum which was a nuisance to those who perfused isolated organs. But with its isolation from blood, and its chemical identifica-

tion, it was not long before it was found to occur naturally in various tissues, including brain. Interest in it was greatly increased, first by Gaddum's observation that lysergic acid diethylamide, a drug producing marked central effects, bizarre behavioural changes in animals, and what is rather loosely described as a schizophrenia-like state in man, is a rather powerful antagonist of 5HT on smooth muscle; and secondly by the finding that reserpine, the widely used principle of rauwolfia, as well as exerting a curious sedative and hypotensive action, is able to rid the tissues, including the brain, of its HT content. *Substance P* is somewhat different and owes its noncommittal name to its more complicated and still unelucidated structure (although it is known to be a polypeptide). It was first discovered by Gaddum and von Euler as an unknown constituent of tissue extracts, occurring particularly in brain and the alimentary tract, and able to contract isolated smooth muscle.

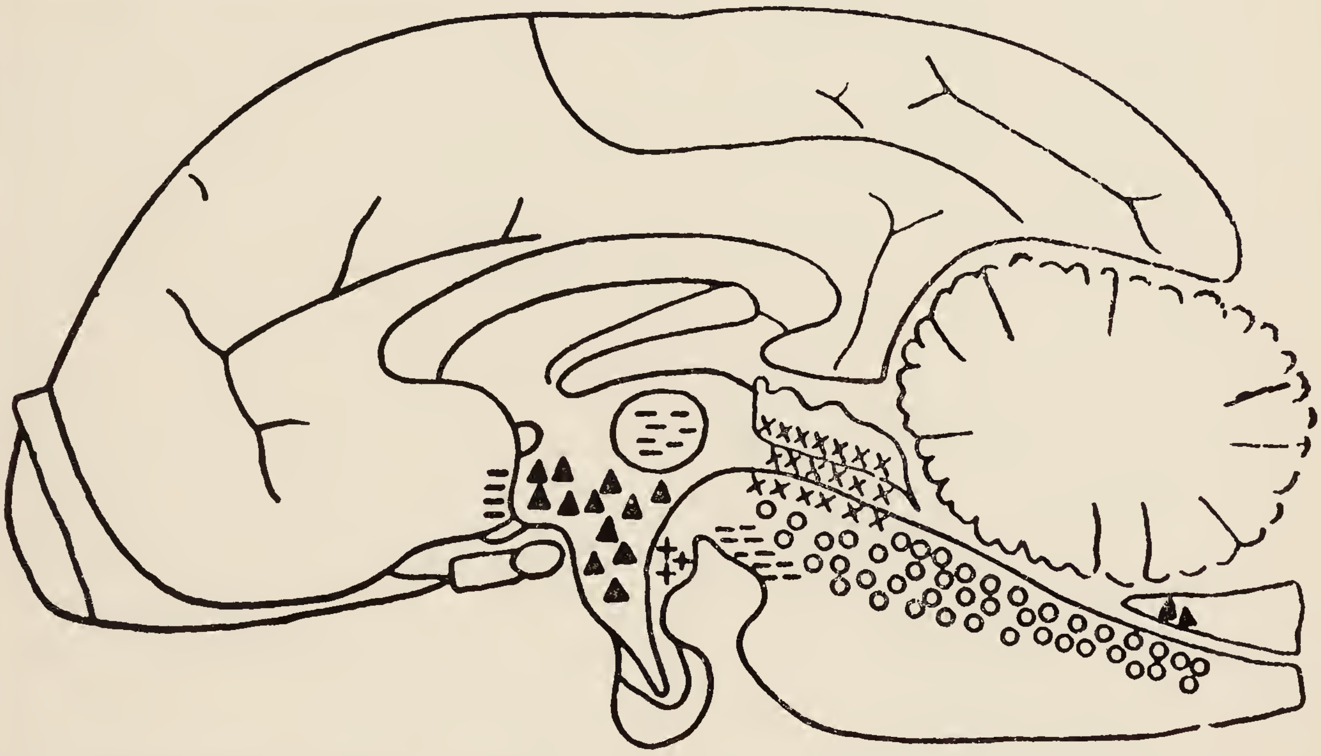


FIG. 2 Medial sagittal section of a dog's brain. Distribution of noradrenaline. ▲, 1·0µg/g; +, >0·4 <1·0µg/g; O, >0·3 <0.4µg/g; –, >0·2 <0·3µg/g fresh tissue. (From Vogt, 1954, by permission of Journal of Physiology).

Distribution and Function. The distribution of sympathin in the brain (FIG. 2), taken from Miss Vogt's beautiful paper[24] on cerebral sympathin, shows not only how noradrenaline occurs in the central nervous system but also serves as an approximate picture for the distribution of Substance P and hydroxytryptamine. With all three substances, their main concentration seems to be round the third and fourth ventricles and aqueduct, perhaps in particular relation to the structure known as the reticular formation in which so much interest is being taken at the moment. Substance P, in addition, occurs in the sensory pathway. Enzymes exist, in the brain, too, which can form

and destroy sympathins and 5HT. Can we say anything as to whether these substances appear when nervous activity is excited in a particular region, or whether they can reproduce normal neural events when administered? As regards the first, one can only refer to Angelucci's finding[1] that fluid which is passed over a frog's spinal cord acquires traces of activity resembling that of HT and that of Substance P, as well as of acetylcholine, when reflex activity is elicited; but the experiments were at the limit of biological sensitivity, and must be regarded as suggestive rather than decisive. When one comes to the effects of administering these substances themselves, there are major difficulties. Substance P has no very convincing central action, whether given systemically or into the C.S.F. (von Evler and Pernow[9]); but this is not a surprising finding in the light of its relatively large molecule, and may reflect a failure of penetration into brain tissue as much as a failure to be active on cells once it reaches them. Adrenaline or hydroxytryptamine have major peripheral actions which may change the sensory input to the central nervous system or even radically alter its blood supply. Given directly to the C.N.S. by ventricular injection, these amines may actually be depressant[14]. An interesting approach has been that of feeding to animals the precursor of 5HT (*i.e.* 5-hydroxytryptophan). This increases the amount of 5HT in the central nervous system; and is said to produce effects similar to those of L.S.D. If an inhibitor of the enzyme destroying 5HT is given, such as marsilid, 5-hydroxytryptophan, in rats at least, becomes still more active. Important as these results are, they are circumstantial in nature, and changes in 5HT content of tissues outside the brain may also contribute to the effects seen. The interpretation of reserpine's action too, demands caution, particularly since Muscholl and Vogt's[22] discovery that it mobilises not only HT but also sympathins. Reconsideration is taking place, too, of the action of drugs like amphetamine or mescaline; for long these have been usually regarded as sympathomimetic, so that their actions could be regarded as indicative of the action of adrenaline centrally. But Dr Vane in the Pharmacology Department here, in an extended study of such amines, has found that they seem as closely, possibly more closely, related to HT. One gains the impression that both the sympathins and 5HT, and possibly Substance P as well, have a central role, but that the evidence points less to any specific synaptic actions than to some general modulator and diffuse action. Perhaps the observation by Bülbring *et al.*[2] that gliomas contain noradrenaline, adrenaline and amine oxidase may be an important pointer – suggesting that glial supporting tissue containing these amines plays a background role in synaptic transmission in addition to its other functions. But for our immediate purpose, none of this evidence has yet furnished

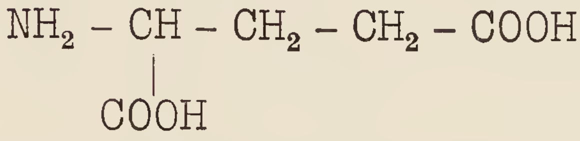
unequivocal proof that these substances are involved in the transmission of a signal from one neurone to another.

(*c*) *γ-amino butyric acid.* This is a new actor on the cerebral stage which appeared when E. Florey found that certain brain extracts could inhibit the discharge of a sensory receptor – the stretch-receptor of the crayfish. This may seem a peculiar test-object; it is however a nerve cell whose responses can be easily and quantitatively recorded and which can be readily exposed to the drug simply by dropping test fluid on to it – a vastly more convenient screening method than any available test on mammalian neurones. The inhibitory factor (or Factor I as it is called) has been shown to consist, in part at least, of the quite simple molecule γ-amino butyric acid, usually abbreviated to GABA, an aminoacid molecule differing from the familiar amino-acids such as alanine only, but significantly, in the separation of the basic and acidic groups.

GAMMA-AMINO BUTYRIC ACID

$NH_2-CH_2-CH_2-CH_2-COOH$

GLUTAMIC ACID



$$NH_2-\underset{\displaystyle COOH}{\underset{|}{CH}}-CH_2-CH_2-COOH$$

FIG. 3 Structural formulae of γ-amino butyric acid (GABA) compared with that of glutamic acid.

The actions of GABA are now being intensively studied by a number of neurophysiologists. Florey and McLennan[12] obtained evidence that it could modify spinal reflexes as well as transmission in some ganglia, but to obtain their results they had to apply it locally to the exposed spinal cord, a method of study which is somewhat unsatisfactory. Eccles and his colleagues[4], failed to observe any effect on spinal reflexes from the intra-aortic administration of Factor I, but this failure may be due to an inability to pass the blood/brain barrier, Purpura *et* al[23] find that it can interfere with cortical responses if applied locally or if given under conditions which they believe break down the blood/brain barrier. This neurophysiological work is still in progress, but there seems no doubt that GABA can have a depressant effect on nervous tissues. The initial idea that it produces direct inhibition is perhaps giving way to the idea that its action is to antagonise excitatory responses. So far one might perhaps not be very impressed with the story; but there is some other information which makes a rather temptingly attractive picture. GABA is

known to occur naturally in the brain and it accumulates in fluid collected from the surface of the cortex. It can be formed in the brain from the decarboxylation of glutamic acid by glutamic decarboxylase. Recently an examination was made by Killam and Bain[19 20] of the ability of a substance used as an enzyme inhibitor, semi-carbazide, to produce convulsions. They found that associated with the onset of convulsions was an inhibition of the activity of the decarboxylase and a reduction in the amount of GABA in the brain. This offered the rather attractive idea that the convulsions were due to a reduction of a normal complement of an inhibitory transmitter; and it accounted, too, for the latency in onset of the convulsions. We do not know yet whether particular cells form GABA, or whether activity in particular cells is associated with synaptic release of GABA. One may, indeed question whether GABA is *itself* likely to be a transmitter. If it accumulates under normal conditions, in cortical fluid, then the cortex must be more or less bathed in it, a notion difficult to reconcile with the activity of normal cortex, if GABA is of high potency. One is uncertain, too, whether GABA could be removed fast enough to play a synaptic role since there is no known means of destroying it rapidly, although Elliott[8] finds that it is rather rapidly absorbed by nervous tissue. But if GABA, say, was to an inhibitory transmitter as choline is to acetylcholine (although no relationship of this sort has yet been established) then much could be explained.

Provisional Summary

Reviewing this field of work, of which I have only mentioned a rather arbitrarily chosen part, one has the impression that acetylcholine is certainly involved in certain central synapses; that noradrenaline hydroxytryptamine and Substance P may well be concerned, but possibly only as modulators; that GABA has a still obscure role of considerable interest on the inhibitory side; but with the solitary exception of the Renshaw cell, we can nowhere say that a definite synapse is operated by a definite transmitter.

INTRACELLULAR STUDIES ON THE SPINAL NEURONES

When the physiologist is confronted with the sort of situation we have outlined, in which the difficulties arise particularly from the complexity of higher central nervous pathways, and the difficulties of recording explicitly from known cells, an approach to some simpler situation is obviously called for. The development of microelectrodes, and such beautiful work as that by Hodgkin and Huxley on nerve, and by Katz and his colleagues at the motor endplate, has proved a strong stimulus to the use of similar methods, and concepts, on the

central nervous system. We owe particularly to Eccles, and his colleagues, Brock, Coombs and Fatt, a fascinating study of the responses of single motoneurones by microelectrodes. I shall outline some of the conclusions he has reached and described in his recent book[6], because it seems to me that it is at this level that many of the facts of chemical transmission may have to be established, now that we know the difficulties and complexities of trying to do it on larger masses of nervous tissue.

The excitatory and inhibitory post-synaptic potentials

If a motoneurone is impaled and a sensory nerve in connection with it is stimulated, then after a short latency from the stimulation one of two responses can be seen. First is what Eccles calls the EPSP, or the excitatory postsynaptic potential. This is analogous to the endplate potential recorded at motor endplates, and is a graded depolarisation of the motoneurone membrane due to the activity of the presynaptic

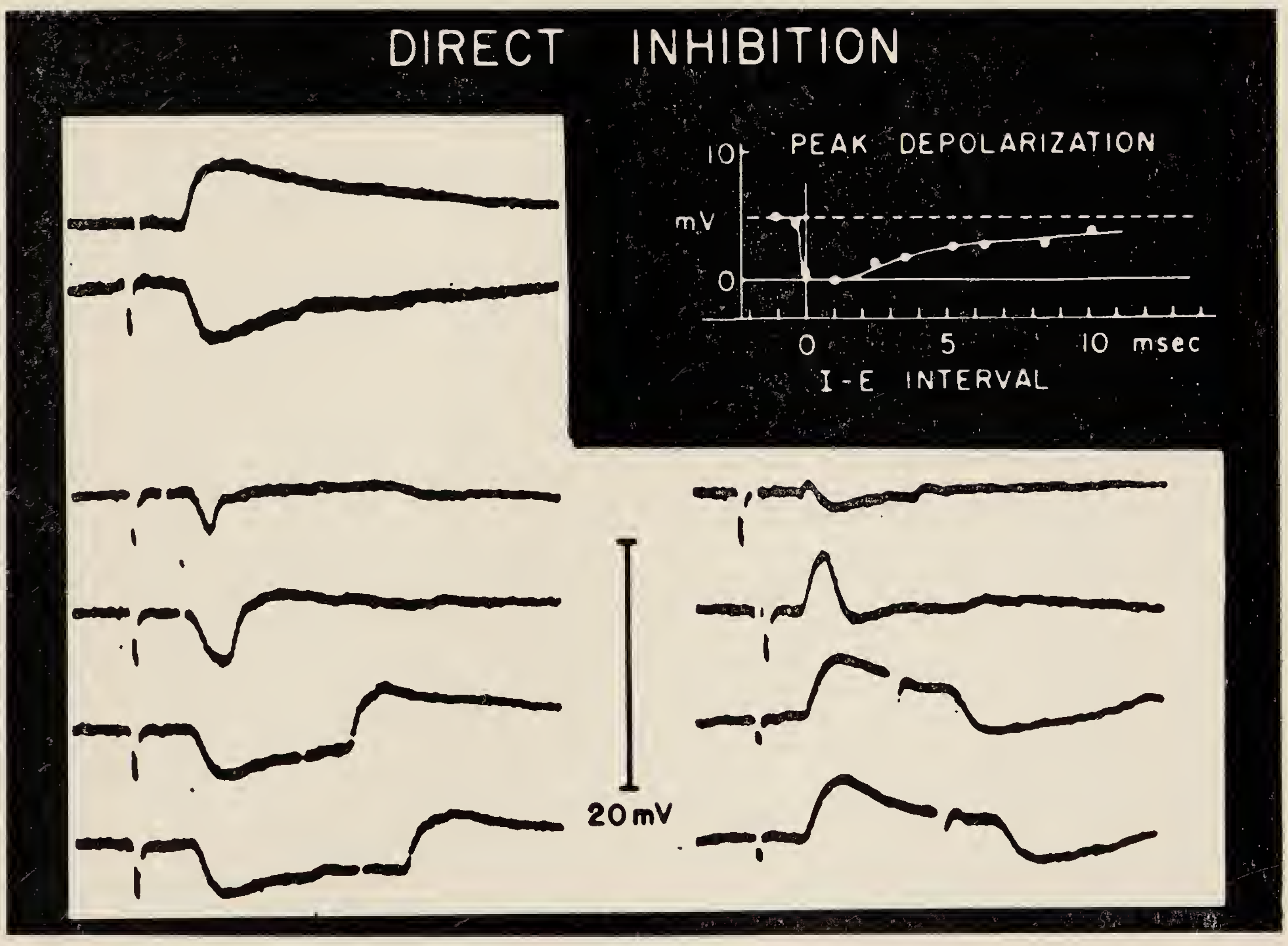


FIG. 4. Intracellular records from a motoneurone of cat spinal cord. In the top left are an excitatory postsynaptic potential (EPSP) above, and an inhibitory postsynaptic potetial (IPSP) below, each pure. The lower half of the figure shows how the potentials interact when elicited together at various intervals. Top right is a graph summarising the effect of the inhibitory volley, given at various intervals before and after the excitatory volley, given at various intervals before and after the excitatory volley, on the peak depolarisation achieved by the latter. Upward deflection signifies membrane depolarisation (internal electrode relatively more positive) (By courtesy of Dr K. Frank).

nerve terminals. If this excitatory potential reaches a sufficient height, then the whole nerve cell is fired off, and one records a full all-or-nothing spike, analogous to the action potential of muscle or nerve. During the spike the polarity of the membrane is reversed briefly; then the membrane potential returns towards its original level, may become more negative than before in an after – potential and finally returns to the resting state. We have then three responses, the excitatory potential, the spike and the after-potential.

Such a response occurs if the nerve stimulated would give rise to a contraction of the muscle fed by the motoneurone, *e.g.* if the nerve was from the muscle spindles of a synergist muscle. If, however, a sensory nerve from an antagonist muscle was used, then a different response is recorded, the IPSP, or inhibitory postsynaptic potential. This is a hyperpolarisation of the membrane, a change of potential in the reverse direction to that of the EPSP, though otherwise rather similar. The inhibitory potential and the excitatory potential, roughly speaking, sum together algebraically; so that there exists a rather elegant basis for the concepts developed by Sherrington of central excitatory and central inhibitory states which could interact and neutralise each other.

Evidence for chemical transmission

Thus far, there is nothing that I have said which necessarily makes one postulate a chemical transmission process rather than an electrical

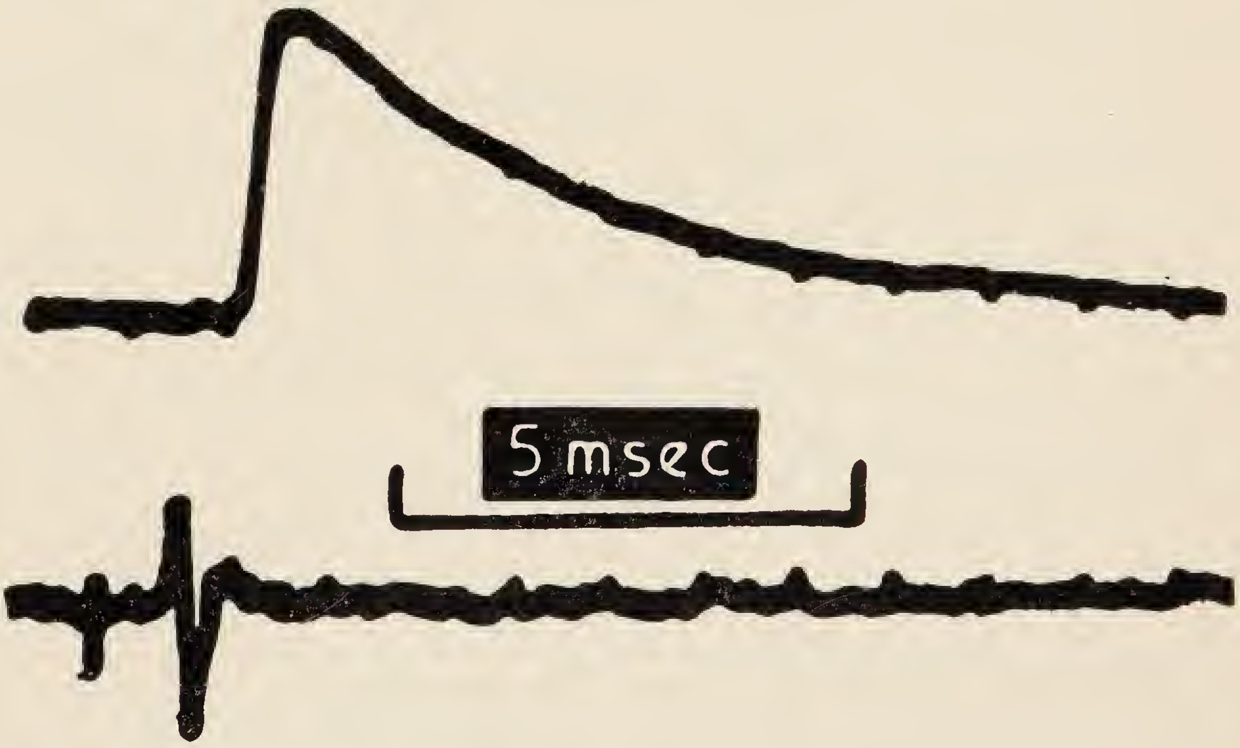


FIG 5 Upper trace: intracellular record of the excitatory postsynaptic potential set up in a gastrocnemius motoneurone in response to excitation of the muscle nerve.
Lower trace: record of incoming volley in dorsal root, to which the motoneurone responded.
(From Brock *et al.*, 1952. J. Physiol. 117, 431).

one. But it was his studies of these responses which converted Eccles from electrical to chemical transmission. Take the excitatory potential first. On the electrical theory, qualitatively, its presence offers no difficulty. It was believed that activity penetrating the presynaptic nerve terminals could cause a negativity at the endings which would be imposed on the motor neurone and thus depolarise the membrane. But the electrical theory fails on the quantitative side. The appearance of activity in the terminals can be detected by an electrode in the vicinity. When the electrode was placed inside the cell, this disturbance can still be recorded; but it is now seen, firstly, to be far smaller than the excitatory potential which can now be recorded, and secondly, to precede it by a distinct latency. Consequently Eccles now believes that the amount of electrical energy available at the endings is too small by far to account for that mobilised during the excitatory potential, and that the timing is wrong.

A second reason for abandoning the electrical theory was the direction of the inhibitory potential. Originally Eccles had put forward a theory (the Golgi cell hypothesis) which by assigning certain special functions to an adjacent Golgi cell, postulated that an axone from that cell became positive (by acting as a source for current flow in its body) and imparted this positivity to the cell membrane. Now with such a mechanism, there should be lines of current flow through the neurone such that at the point where this axone was applied to the

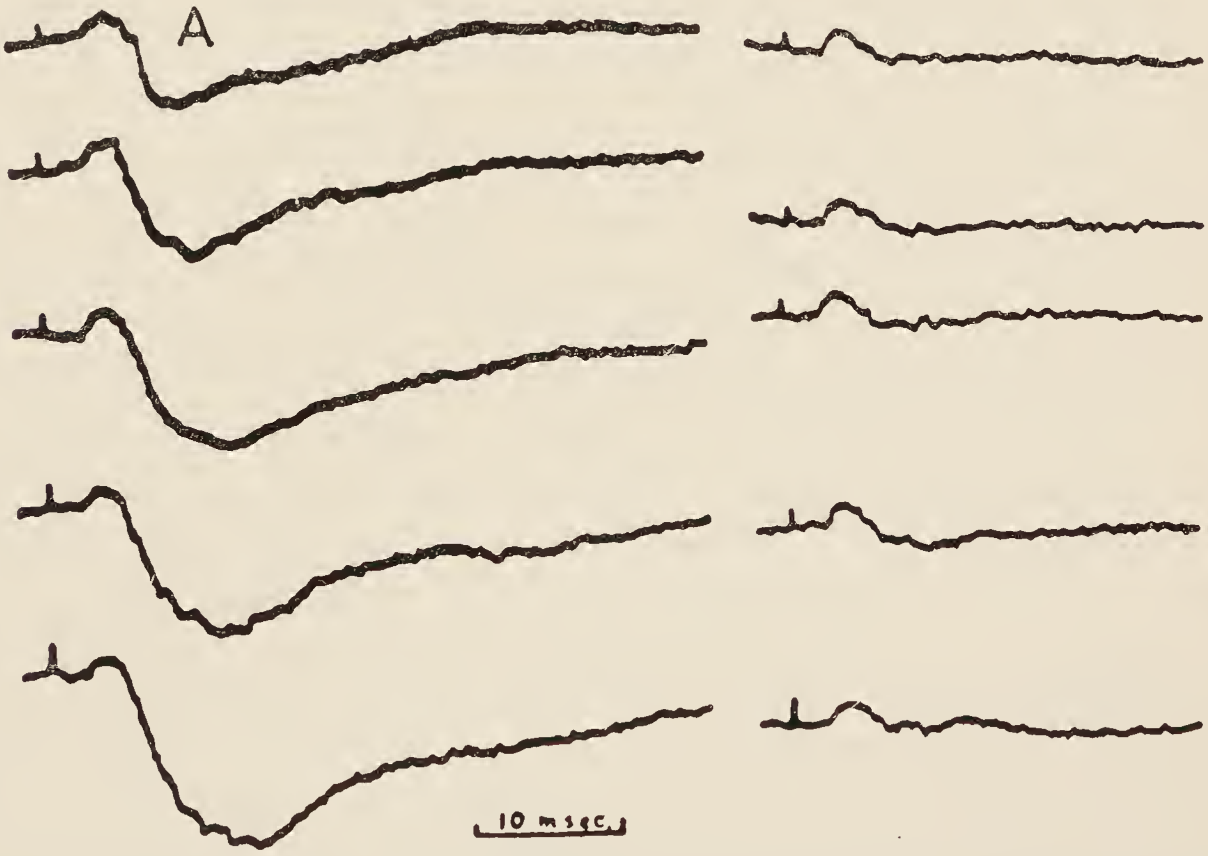


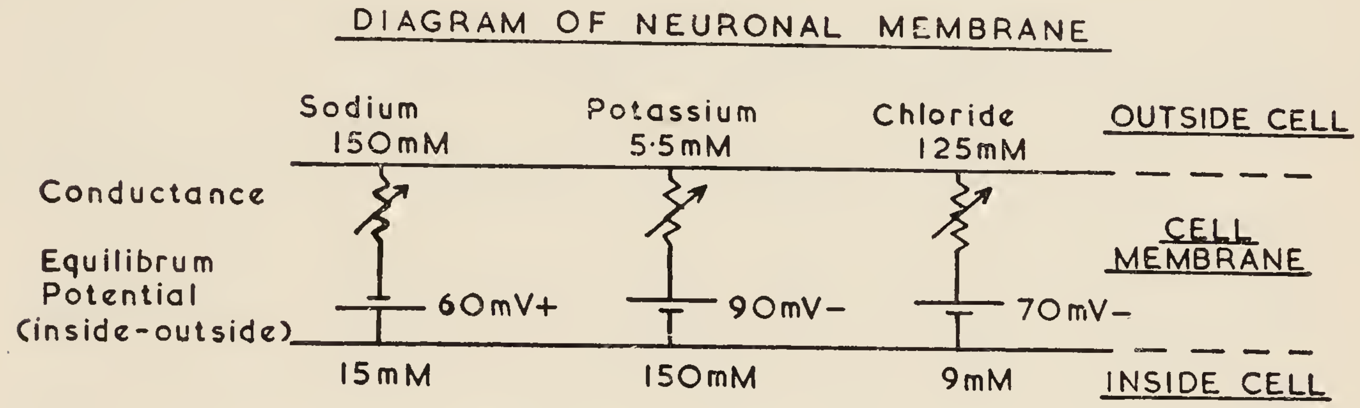
FIG 6. Potentials recorded from a gastrocnemius motoneurone in response to inhibitory volleys (sural nerve stimulation) of increasing strength. A: potentials recorded intracellularly B: potentials recorded under identical conditions, but just outside the motoneurone. (From Eccles, J. C. (1953). The Neurophysiological Basis of The Mind. Oxford.)

nerve cell, the membrane would be hyper-polarised, but that at the regions of the nerve cell where the current flowed out of the cell again, there should be a depolarisation; and with an electrode placed inside the cell one would expect to see either no substantial potential change or a small internal positivity relative to an indifferent electrode, corresponding to the somewhat positive field in which the whole neurone was placed. What is actually seen is an internal negativity much larger than the externally recorded potential; and, of particular significance, the potential reverses as the electrode penetrates the cell, showing that it is generated at the cell membrane, and not outside it (FIG. 6). A further important point arose here. As I shall mention later, these excitatory and inhibitory potentials vary both in their magnitude and in their sign if the membrane potential is shifted artificially. This is even harder to explain on any electrical theory; for on that assumption the sign of the electrical change should be the same whatever the membrane potential, although a change in membrane resistance might alter the magnitude of the potential recorded.

THE MECHANISMS BY WHICH THE POTENTIALS ARE PRODUCED

To analyse the means by which the various potentials are described and produced Eccles relied to a large extent on methods developed in recent work on nerve physiology; and I would like to do no more than present the picture offered by him in his book. This is rather neurophysiological, but the final picture of how the potentials arise is fundamentally a simple one, even if the steps to it are not. We have first to distinguish between the potential across the resting membrane and the potential changes which occur when various transient phenomena take place. The resting membrane is polarised so that the inside is approximately 70mV negative to the outside. This potential depends on the presence of negative charges inside the cell which are not diffusible but which, to achieve electrostatic neutrality, attract a corresponding number of positive charges and repress entry of negative ions. The nature of the positive charges entering is conditioned by the exclusion, from the resting cell, of sodium. Potassium and chloride, however, are relatively free to move, so that they become relatively concentrated in or excluded from (respectively) the inside of the cell. To be exact, Eccles believes that the charge across the membrane and the difference in the concentration of potassium either side are not in exact equilibrium, potassium being, to a small extent, pumped continuously into the cell. Chloride ions, on the other hand, he believes to be in equilibrium, *i.e.* that the concentration difference is exactly balanced by the potential difference, tending to move them in the other direction.

So much for the resting state. FIGURE 7 summarises the position; a lot of sodium outside, a lot of potassium inside and a relative preponderance of chloride outside. I must explain here that the diagram expresses the way that the uneven ionic distributions are equivalent to a set of batteries, yielding potentials depending in size on the ratio of ionic concentrations, and in sign on the direction of relative preponderance. The extent to which each battery can operate, *i.e.* the current carried by it, depends on how permeable the membrane is to the ion concerned, *i.e.* to the conductance for that ion; so that one can represent permeabilities by a variable resistance. If the permeability is very low, *i.e.* the resistance is very high, then that battery can hardly pass any current. Thus the potential at any moment is going to depend both on the voltage of the batteries and on the resistance in series with them. Now suppose we make the membrane suddenly permeable to all these ions, a process which Fatt and Katz showed to exist at the motor endplate under the influence of acetylcholine, (although they were careful to point out that they had shown this to be true only for the ions they were able to study) *i.e* suppose that the resistances in series with these are all brought low so that all the batteries can work. Then the membrane will approach an intermediate potential repre-



CONDUCTANCE CHANGES DURING ACTIVITY

1) E.P.S.P.	Increase	Increase	Increase	["Short circuit," all ions]
2) I.P.S.P.	—	Increase	Increase	["Small ions" only]
3) SPIKE RISE	Increase	—	—	[Sodium only]
4) SPIKE AFTERPOTENTIAL	—	Increase	—	[Potassium only]

RESTING STATE: 70mV— inside

[Sodium extrusion
Potassium intrusion
Chloride equilibrium]

FIG. 7. Diagram showing above the estimated ionic concentrations outside and inside the motoneurone, and the equilibrium potential resulting for each ion; below, the changes in conductance which have been proposed for various motoneurone responses.

senting the balance of the action of all the batteries, somewhat on the negative side of zero, and representing a considerable reduction of the normal membrane potential. This is the situation which Eccles believes occurs when an excitatory potential is set up, as with the activation of the endplate by acetylcholine.

Suppose, on the other hand, that a great permeability increase takes place to one ion only, say sodium. Now its equilibrium potential will dominate the picture, and swing the membrane potential to the reverse of normal – as happens in the rising phase of the spike.

One may well ask how this model is substantiated. Besides recording the potentials described, and making measurements or estimates of ionic concentrations, two special types of experiments are made. First, one may change the membrane potential artificially, and then determine what happens during one of the responses described. It is then found that if you carry the membrane, say, to +30mV inside, the EPSP now reverses in direction; and you can find a membrane potential, called the 'equilibrium potential', at which *no* active response is recorded. This is regarded as measuring the membrane potential which just balances out the ionic batteries physiologically activated in the response considered. The second type of experiment is to inject different ions inside the cell: clearly one will change the composition of the battery, and hence the potential of which it is capable. A striking example is that of introducing chloride ion into the cell; enough indeed diffuses out of the micropipettes often used (KCl filled) to produce the effect; it rapidly reduces the IPSP, and may even cause it to reverse. Such a result conforms with the model; the Cl ratio falls, the Cl battery becomes less effective; and the mean potential derived from the chloride and potassium ions will fall, first to the normal resting membrane potential (so that the IPSP normally recorded vanishes) and then beyond it (so that the IPSP reverses).

A good deal of the picture presented will require revision. But it seems highly likely that the mechanism discussed, of potentials dictated by ionic concentrations, by the electrical forces moving ions, and by the effects of transmitters on permeability, will be the elementary physiology of tomorrow's medical student. In a sense the final model is a very easy one; one can simply picture a membrane containing holes, pores or channels, whose dimensions are sensitive to transmitters or membrane potential. Thus the problem shifts from 'What is the source of these potentials' to 'What controls membrane permeability to ions'. Oversimple as such a model will prove, it is still fundamental to any pharmacological study of transmitter action.

There is one further response, which ought to be mentioned, described first by Frank and Fuortes[25]. So far, I have spoken as though when a given motoneurone is inhibited, an obvious hyper-

polarisation develops at its membrane. But Frank and Fuortes found that it is possible to get quite a vigorous inhibition of an excitatory reaction although when the inhibitory volley alone is administered, there is no potential change in the cell (FIG. 8). This they call 'remote inhibition' .It is clearly an important inhibitory mechanism, often accompanying an inhibitory volley, and having sometimes an appreciably longer time course than the ordinary direct inhibition. It presents a third synaptic process for which a transmitter must be identified.

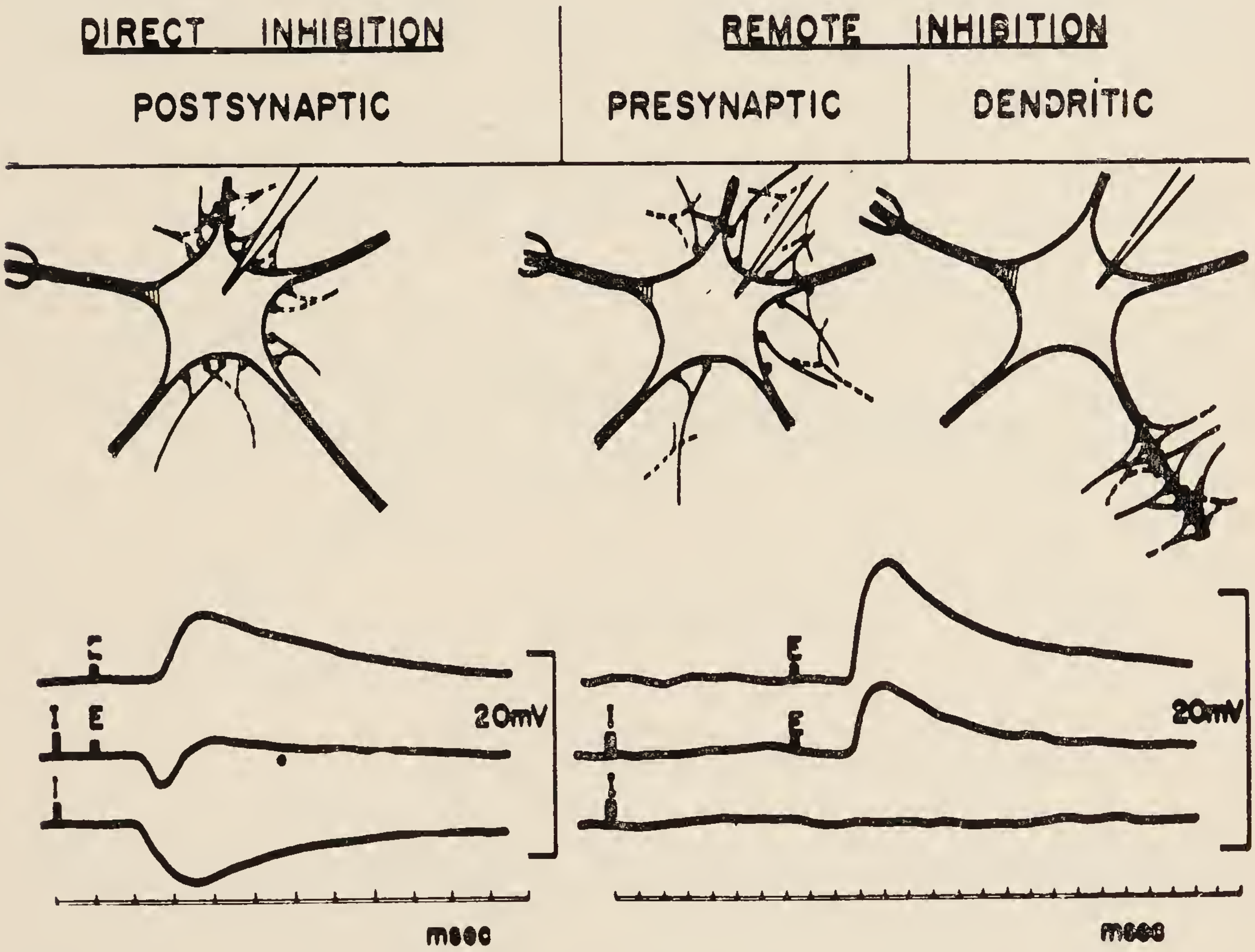


FIG 8. Comparison of 'direct' and of 'remote' inhibition. On the left, are (from above down) the response to an excitatory volley, to a combined excitatory and inhibitory volley, and to an inhibitory volley alone; the latter produces a hyperpolarization. On the right are similar records obtained using 'remote' inhibition; the inhibitory volley can reduce the EPSP considerably, yet produces no potential change itself.

The upper part of the figure represents diagramatically ways in which the inhibitory effects might be brought about. (By courtesy of Dr Karl Frank).

Studies with drug administration by the concentric electrode

Given all this new information, the ground is laid for a new attack on the nature of the central transmitter, if one could do one additional thing, that of delivering test drugs locally to individual motoneurones. This would give the following advantages. (1) The drug would be applied to a single neurone, and not to a whole mass of intertwined and interacting cells, (2) The blood brain barrier is by-

passed. (3) Such a technique would allow direct comparison of the effects of the drug on the motor neurone with normal synaptic events. It was with these considerations in mind that I have recently been working with Dr Frank in his laboratory in the National Institutes of Health. Our object was deceptively simple; to find something that would stimulate a motoneurone, in the hope that it would be a pointer to the natural stimulant. Although our experiments were not as fruitful as hoped, and are in fact still being analysed, some details of the method used by us may be of interest. The opportunity to do this work was presented by Dr Frank's development of a concentric micro-electrode (FIG. 9). Here a fine inner micro-electrode is nested inside a slightly larger one and allowed to protrude a little bit beyond it. If then, one could penetrate a motor neurone with the inner fine electrode and leave the outer, slightly wider bore, electrode outside the motor neurone, then one would be able to record the response of the cell with great sensitivity with the inner electrode, and deliver drugs to the surface of the membrane through the outer.

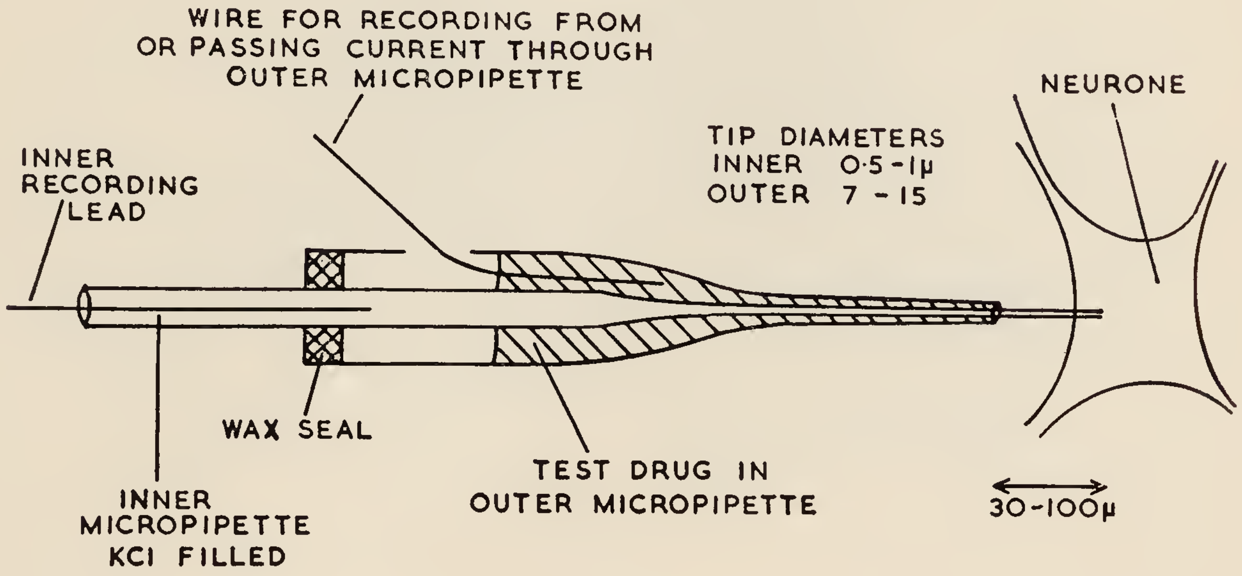


FIG. 9. Diagram of concentric microelectrode.

To deliver the drug various means might be proposed. One might, for instance, simply try to squirt it by pressure. This offers some difficulties, since one needs a completely enclosed system, one may need fairly high pressures, and one might well push the motoneurone off the inner electrode. A more serious objection arises from temperature effects; water expands faster than glass with rise of temperature; and to prevent the movement of fluid in and out of the pipette from temperature change, would require control of temperature to 0.01°C. or better. The method we used was that of iontophoresis, a method which had been used very successfully by Nastuk, and by Castillo and Katz for stimulating the motor endplate with acetylcholine. The main difference was, of course, that they had used electrodes of about

1μ or less in diameter, while our outer electrode had a diameter of 7–15μ. The principle of iontophoresis, of course, is simply that you apply a potential to the drug solution and pass current between it and the rest of the animal; if, for instance you make the outer electrode positive, then any positive ions in the solution will tend to be expelled from the electrode.

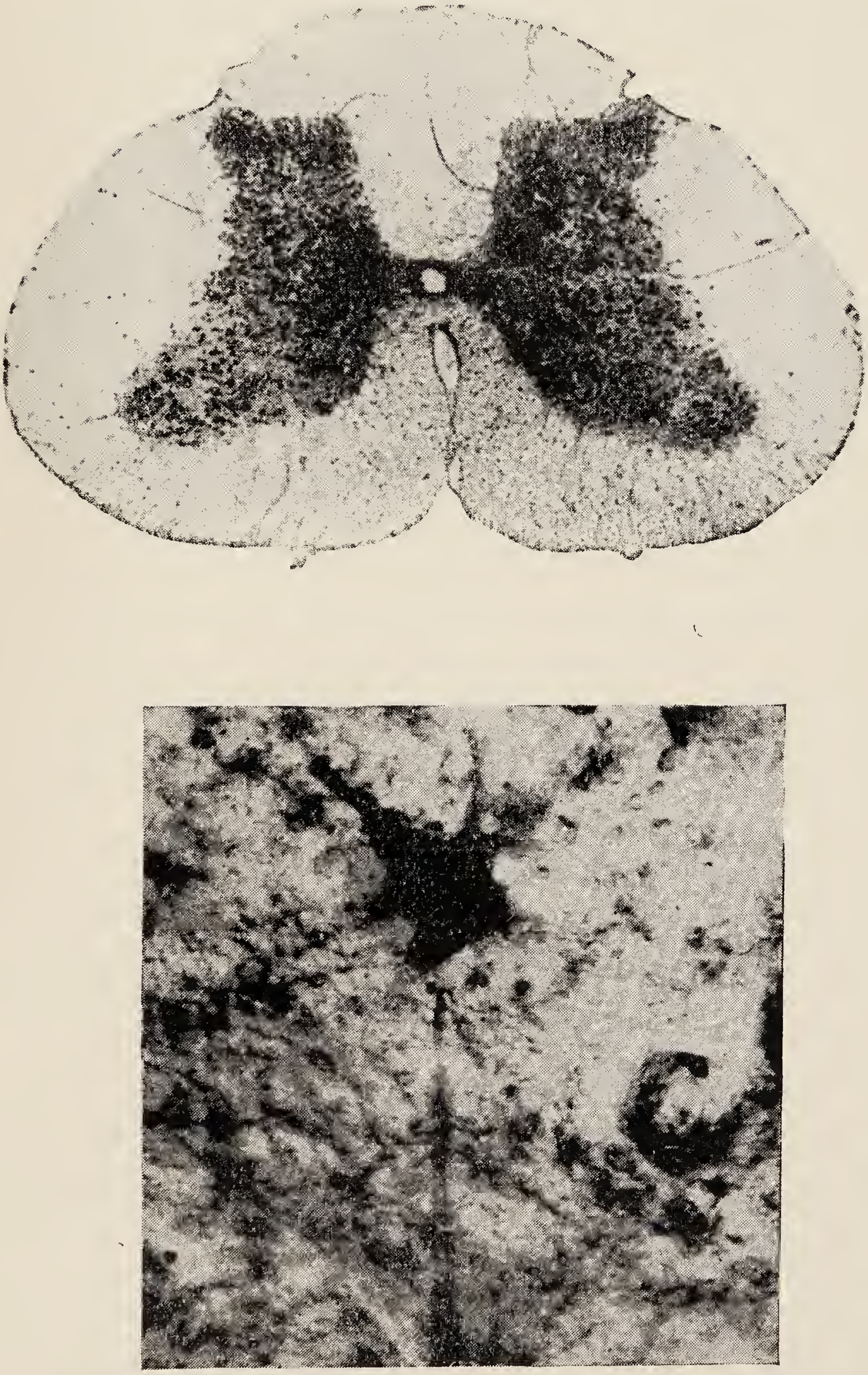


FIG. 10. Above, section of cat's lumbar cord. Below, motoneurone in a thin slice of cord, with micropipette inserted, to show general scale of structures. (By courtesy of Dr Karl Frank).

The procedure in our experiments, then was roughly as follows. First of all anæsthetise the animal, cut the spinal cord and destroy the brain (to minimise descending background influences and to make anæsthesia unnecessary); then expose the lower part of the spinal cord by a laminectomy, identify the roots and mount them on electrodes, and render the whole preparation as rigid as possible, curarise the animal and place it on artificial respiration. Then the

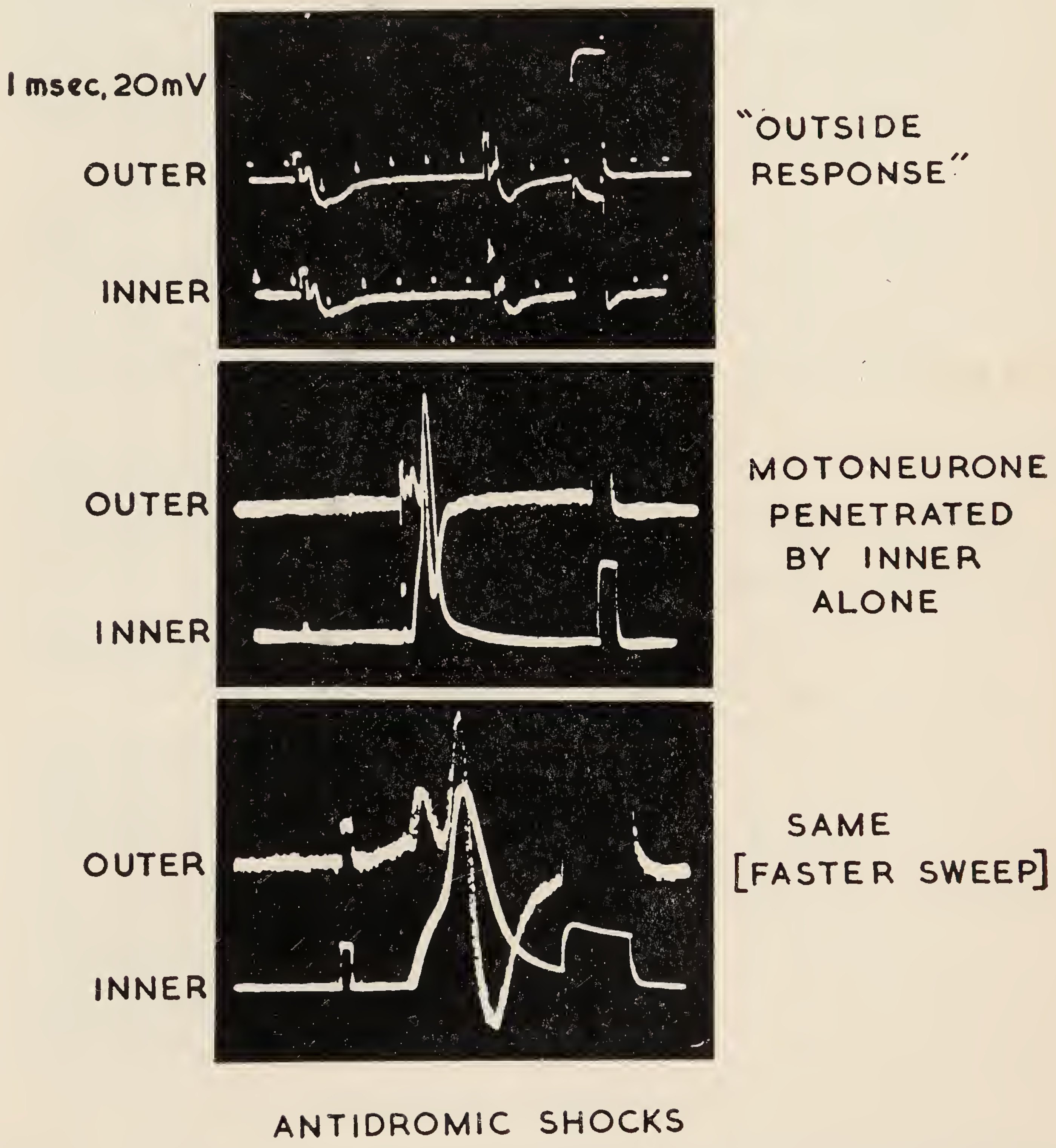


FIG. 11. Upper part; responses, recorded by both inner and outer electrodes, of motoneurone pool excited antidromically, and recording extracellularly.

Middle part; the inner electrode has entered a motoneurone, but the outer is still outside. In response to the antidromic shock, the inner electrode records a typical spike action potential. The outer electrode, at higher amplification, records only a diphasic response.

Lower part; same as middle part, but at faster sweep speed.

Calibration: for both electrodes, the partly rounded square wave at the end of the traces is 20mV high and 1msec. long.

concentric micropipettes are mounted and introduced into the cord, descending vertically through it until a region of motoneurones was discovered. The standard way of doing this is to stimulate the ventral roots antidromically; then, when one approaches a motoneurone pool, at a depth usually between 2 and 4mm below the surface of the cord, in the right longitude, one sees what is called the 'outside

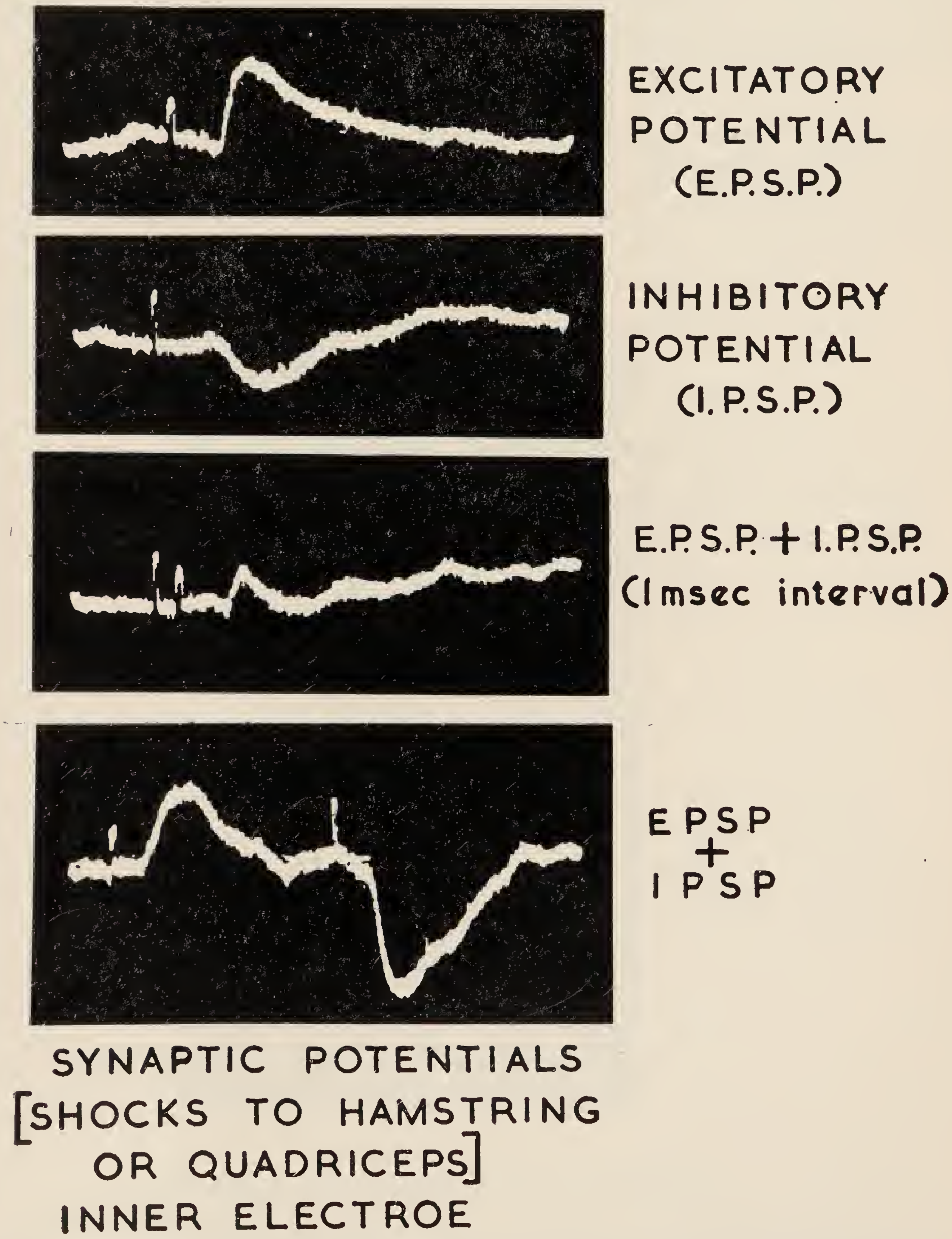


FIG. 12. Records of postsynaptic potentials (excitatory volleys from hamstring muscle nerve stimulation; inhibitory volleys from quadriceps muscle nerve stimulation) in a biceps-semitendinosus motoneurone, recorded with the inner electrode of a concentric microelectrode arrangement.

response', a potential which looks somewhat as in FIG. 11. At this point one slows up one's advance and begins to try to penetrate a motoneurone. If one is successful, the entry is signalled by a large shift in potential of the inner electrode to the membrane potential of the neurone; and in response to the antidromic shock a large spike is usually seen. This will allow one to test the effect of a drug on the antidromic response, which essentially is the study of the properties of the motoneurone as regards its spike discharge and the ability of an impulse to invade from the motor axone over the whole body of the cell.

Since, however, it is synaptic events which are of primary interest, our next task is to see whether excitatory and inhibitory potentials can be recorded. To do this the best motoneurones for practical purposes to use are usually of the biceps-semitendinosus group. One now

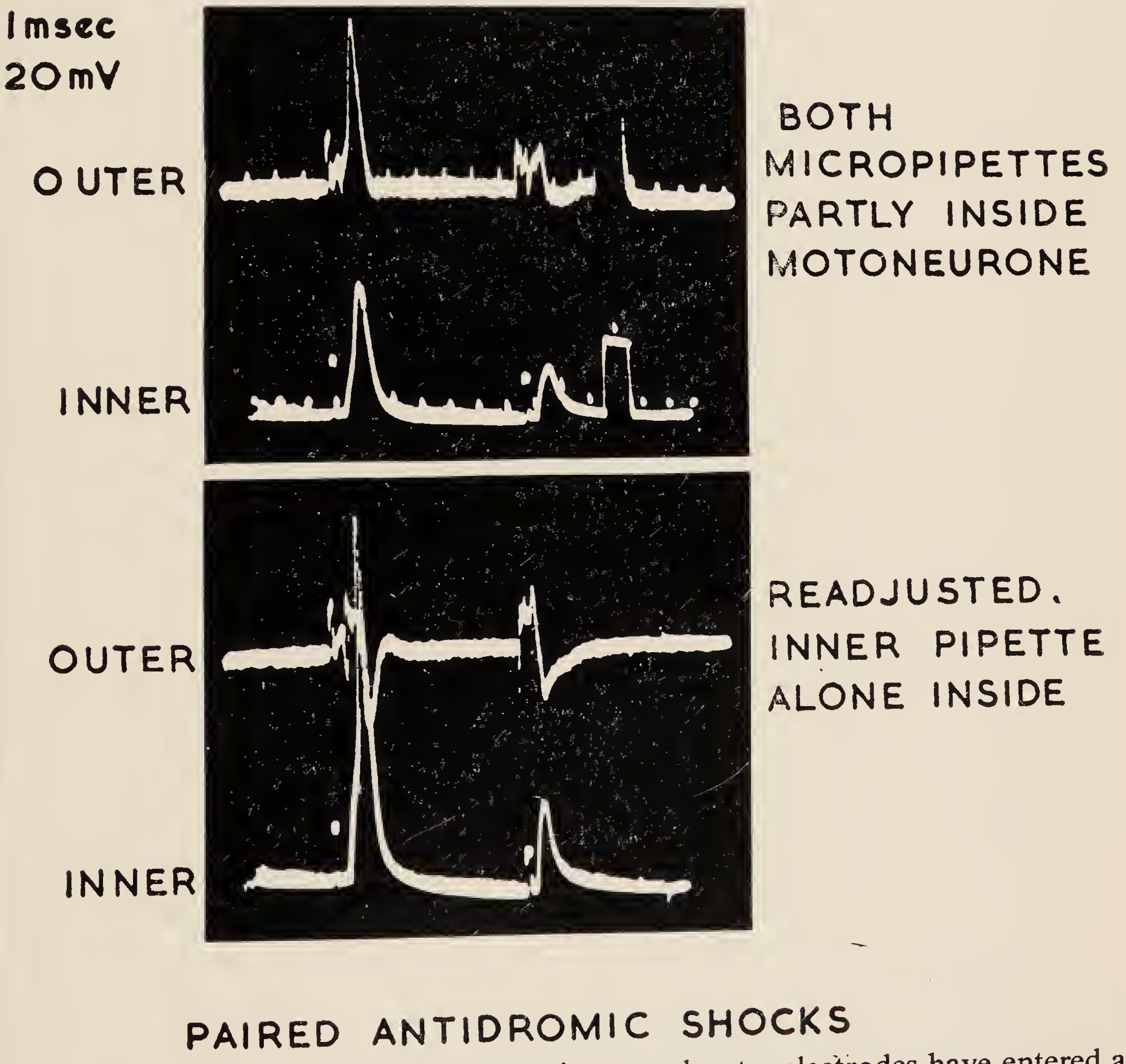


FIG. 13. Upper pair of records: both inner and outer electrodes have entered a motoneurone, and spikes are recorded in response to antidromic excitation.

Lower pair of records: the electrodes have been withdrawn a few microns; now only the inner electrode records a spike (larger, since current leak through a large hole in the membrane is now reduced).

stimulates muscle nerves, to test whether the motoneurone will respond (for instance) with an excitatory potential to hamstring stimulation, and with an inhibitory potential to quadriceps stimulation (FIG. 12). All this time it is necessary, of course, that the inner fine electrode should keep inside the motoneurone and the outer one outside (FIG. 13). If we have succeeded so far, then one chooses one or two of these possible responses, and tests whether it is modified by iontophoresis of the drug in the outer electrode. Here a major trouble has been that the passage of current alone can apparently shift the relative position of motoneurone and electrode.

The results of the experiments are still, as I have said, under analysis. But for the purposes of this talk, I have to report that we failed to produce any really effective activation of a neurone. Such small changes in the threshold or spontaneous firing that we saw could be attributed only too easily to tickling the cell membrane by small movements of the electrode. Now, despite the limitations of time and the difficulties of technique, we were able to test nearly a dozen drugs. In choosing them, we accepted the evidence scattered in the literature that histamine, noradrenaline and hydroxytryptamine could not excite the motoneurone, and sought first for drugs with an established ability to stimulate the spinal cord, then for substances able to excite nervous tissue one way or another. These included well-known analeptics like metrazol, nikethamide, strychnine, acid fuchsin, daptazole, and ammonia; penicillin (known to excite caudate cells); barium (known to excite ganglia); phenyl biguanide (known to excite certain nerve endings); guanidine (known to produce activity of excitable tissues); and potassium and acetylcholine. In using acetylcholine, of course, we were aware of the evidence that it cannot activate motoneurones; but we wished to verify this ourselves, and to see whether perhaps other structures could be shown to respond. We also tested GABA, to examine the nature of its depressant action. To the best of my knowledge nobody knows what ion we ought have to put in to the pipette. But I have the feeling that we ought to have been able to excite the motoneurone with one of these, when one considers the range of pharmacological actions, and diversity of chemical structures displayed by them. Our failure, in fact, may itself be a significant result, about which one ought to think seriously.

One problem, of course, is to know whether we are really delivering the drugs, and it is difficult to check this. If one does *in vitro* experiments with this method one immediately finds that the physics of micropipettes is somewhat complicated. There is continuous leakage of drug from the tip by diffusion to the outside; this might well mean both that there is a continuous modest application of the drug as one works through the cord, and that there might be a reduced output at

the moment when one wants to give it. Further, although these microelectrodes are small, they are not so small as to be quite immune to the effects of hydrostatic pressure and capillarity. Finally, we encountered the phenomenon, with which we should have been more familiar, known as electro-osmosis. This arises from the possession by a glass surface of an electrical double layer; such a surface in water, or saline, or plasma, becomes negatively charged, and the fluid phase in contact with it acquires positive charge. If a potential is then applied between the two, the fluid phase is moved relative to the fixed phase; this movement is called electro-osmosis and can be quite

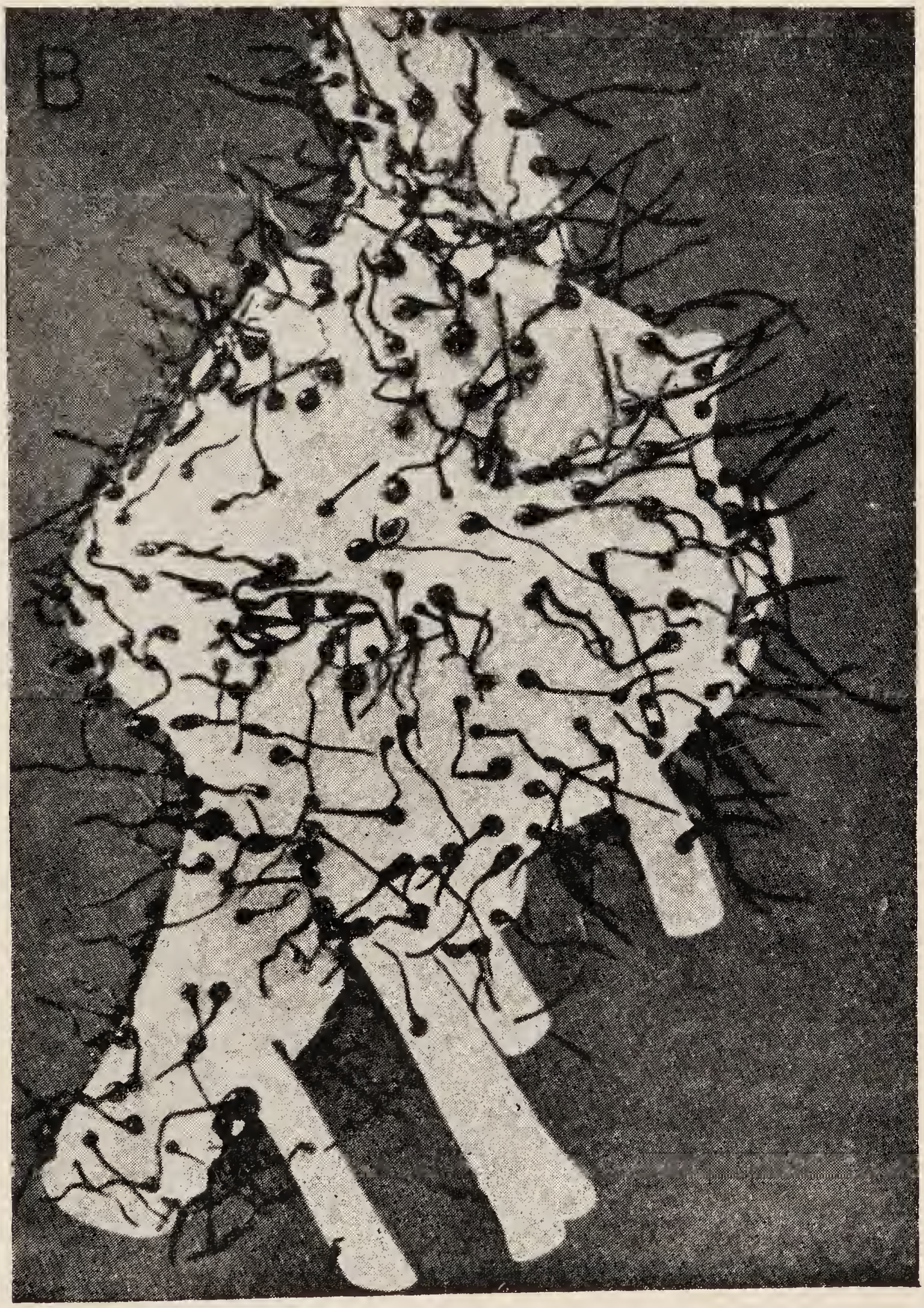


FIG. 14. Model of soma and large dendritic stumps of a mammalian motoneurone, showing dense population of synaptic knobs. Constructed from serial sections (from Haggar and Barr 1950. *J. Comp. Neurol.*, *93*, 17).

substantial. Our *in vitro* experiments, however, led us to believe that we were delivering drug; and we were able to verify directly with acid fuchsin, a powerful red dye, that we could deliver the dye to the surface of the cord by iontophoresis.

Considering our results, therefore, it seems probable that in fact the drug was successfully delivered, and that on a good many occasions we were in a position to deliver it near the surface of the motoneurone.

What other reasons for failure might there be? This brings me to consider the detailed structure of a nerve cell. One's conventional picture of it is shown in FIG. 14; one can see what are known as *boutons terminaux*, beneath which one used to imagine receptor membrane existed. On such a picture as this one cannot see any real reason why a drug should not be fairly sure of reaching receptor membrane. There is, however, one point which immediately arises. Is it possible that we are delivering too localised an application of drug? It is said that *boutons* are spread fairly diffusely over the whole motoneurone. But the way we are delivering it would give a fairly concentrated application over an area of 20 or 30μ diameter, and then a rather rapid fall away of concentration in more remote regions. If the normal synaptic processes involve a diffuse activation of membrane, then we may be doing quite the wrong thing and not getting a wide enough application of transmitter to be effective.

But another point arises, if one considers the more recent studies of the outer structure of the neurone as shown by electron microscopy. Professor Causey has kindly shown me some of the microphotographs made by Dr A. A. Barton in his department; these are, indeed, of ganglion cells which are not, of course, identical with motoneurones; but as far as we know they exhibit similar features. Two points emerge from considering the intimate structure of such cells. The first is that every cell is enshrouded in a satellite cell; so that if drug is to reach the neurone, it has to penetrate the limiting membrane of the satellite cell before it can reach the neuronal membrane. Nobody really knows what the permeability characteristics of the satellite cells are. One can, of course, say with confidence that they are permeable to *some* extent, since we know that many drugs given by mouth and absorbed into the bloodstream will produce central nervous effects. What we are not so sure about is the quantitative relationships. It is difficult to argue from the concentration of a drug in the blood to the concentration occurring in the region of the satellite cell; and thence to the concentration at the surface of the neurone membrane itself; and finally to compare this with the concentration we achieved. If one assumes all the current we passed was distributed among the ions according to their conductance (which we measured in each case), and made some other

assumptions about the probable distance of the tip of the outer electrode from the neurone, then one would have thought that probably we were administering enough drug. But the whole calculation is fundamentally uncertain.

But there is a second point suggested by an examination of nerve endings in relationship to the neurone. There seem to be two sorts of ending in the vicinity of the neurone. On the one hand there is a very close synaptic union where an axone comes within the satellite cell; then the satellite membrane is actually reflected away so that the axonal membrane and the neuronal membrane come to be almost in direct apposition. This would be a very favourable situation for transmitter action, and even relatively small amounts of transmitter might be able to achieve a very high local concentration in their action here. On the other hand there are also more numerous and larger congregations of nerve endings, still wrapped within satellite cytoplasm, in aggregates on the surface of the neurone, structures which may correspond to the '*boutons terminaux*' of classical histology. Here the relationship of the axone to the neurone is much more indirect and a transmitter released by the axonal ending would have to traverse several membranes to reach the surface of the motoneurone. Now what are the functions of these two types of ending? Is the large aggregate functional at all as a synapse? If it is functional, does the large synaptic gap confer on it synaptic behaviour different from the relatively explosive synaptic potential which we are familiar with at the neuromuscular junction? Could the membrane response to the transmitter when it has finally diffused across this relatively large gap differ from that when transmitter is released directly on to the motoneuronal surface? Is it possible that the satellite membrane of the motoneurone is in fact quite a substantial diffusional barrier ? If this were so then only if we succeeded in getting our pipettes within the satellite cell cytoplasm, or better still actually in an interspace between an axone and neuronal membrane, should we succeed in activating a nerve cell; and to do this, one would have to place one's pipette with an accuracy not of microns but of Ångstrom units.

CONCLUSION

I have tried in this review to present some ideas in current work on central transmission at the expense of a properly balanced critical discussion. We have the studies with tissue extracts and enzymes on the distribution, synthesis, and destruction of possible transmitter substances; the experiments, very difficult, on their release; and the accompanying work on drugs bearing varied relations to possible transmitters. We have surveyed some of the electro-physiological

work, which has strengthened the belief in chemical transmission, has defined some of its electrophysiological properties but has produced no new identified transmitter. We also looked at the problems and results of one attempt to satisfy a simple demand, something to activate a motoneurone. Finally we turned to the microanatomy of the neurone and its synapse, to see what a complex structure it is. It is a tantalizing field; I suppose, putting it bluntly, our attempts to understand anæsthetics without knowing the central transmitters are like trying to interpret curare or eserine before the role of acetylcholine was known. It brings to mind a stage set, a spot light ready, and an audience keenly expectant. All we need is some Joseph Clover of today to persuade the Prince of Denmark to take his cue.

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